

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Charles Henry HORN et al. Confirmation No.: 1275
Application No.: 10/521,847 Group No.: 1651
Filed: November 23, 2005 Examiner: Kade Ariani
For: *Megasphaera elsdenii* Strain And Its Uses

Commissioner for Patents
P.O. Box 1450
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DECLARATION OF JANE A. Z. LEEDLE, PH.D. UNDER 37 C.F.R. §1.132

I, Jane A. Z. Leedle, hereby make the following declaration:

1. I am a Ph.D. microbiologist with extensive experience in anaerobic, gastrointestinal microbiology, animal agriculture, ruminant nutrition and fermentation systems. I have developed new microbial products with proven influence on livestock performance.
2. I have been the president of JL Microbiology, Inc., a consulting firm, from 2005 to the present. Further details of my education and professional experience are summarized in the attached Curriculum Vitae.
3. A current client of JL Microbiology, Inc. is Kemira Phosphates Oy, Mechelininkatu 1a, PO Box 900, 00181, Helsinki, Finland, one of the assignees of the subject U.S. patent application 10/521,847 ("the '847 application").
4. I am the first named inventor of U.S. Patent No. 5,380,525 issued on January 10, 1995 for "Ruminal Bacterium For Preventing Acute Lactic Acidosis" ("the Leedle et al. '525 patent").
5. I have studied the '847 application and the amended claims, and state as follows.
6. It has been known for more than 50 years that *Megasphaera elsdenii* consumes lactic acid in ruminants. The challenge has been to find a *Megasphaera elsdenii* strain with metabolic attributes sufficiently robust not only to utilize lactic acid when available, but also to manage changing ruminal lactic acid concentrations when livestock diets are switched from forage to grain. These

attributes include growth rate, cumulative biomass, pH tolerance and continued metabolic activity at low environmental pH values.

7. Along with many others in the field of ruminant nutrition and health, my co-inventors of the Leedle et al. '525 patent and I were working on the problem of providing such an improved *Megasphaera elsdenii* strain for several years. The results of our efforts are, in part, disclosed in the Leedle et al. '525 patent.

8. In the area of lactic acidosis, researchers have reported many lactic acid consuming microbial preparations with varying degrees of success including direct transfer of ruminal content from a healthy to an affected cow to obtain a cure from lactic acidosis. Direct inoculation is necessary because it is well documented that indigenous *Megasphaera elsdenii* (usually present in the rumen) do not respond fast enough to successfully manage sudden increases in ruminal lactic acid concentrations when diets are changed. Normally, cell division takes too long for indigenous *Megasphaera elsdenii* to increase their population density (biomass) or similarly, to increase their metabolic activity for a timely response to increased substrate availability. Without inoculation of exogenous microbes, lactic acid accumulation adversely affects both short-term and long-term animal performance.

9. Further, it is well known that *Megasphaera elsdenii* strains produce propionate when grown in a pH-neutral (ruminal or *in vitro*) environment. Although propionate is highly desirable as a fermentation end product for ruminant meat and milk production, most indigenous *Megasphaera elsdenii* are inhibited at pH values below 6.0. When diets are changed from forage to grain the increased bacterial fermentation creates lower pH conditions. At these pH values, most indigenous *Megasphaera elsdenii* produce end products other than propionate, exhibit diminished metabolic activity and thus may no longer participate in lactic acid consumption. Lactic acid may accumulate. The negative impact on the animal is both short- and long-term.

10. Despite the many studies and considerable literature surrounding *Megasphaera elsdenii* as a lactic acid consuming bacterium, the foregoing discussion also shows that there was ample room for discovery and improvements in 2002. It is my professional opinion that, through an innovative approach using microbiological screening methods that included the selective pressures of pH-auxostat fermentation, the inventors of the '847 application discovered and characterized a number of strains with metabolic attributes that are microbiologically superior to those of strains previously described in patents and the literature. *Megasphaera elsdenii* CH4 is a strain that has characteristics that stand out

for the following reasons.

11. As disclosed in the '847 application, strain CH4 has a high growth rate, 0.93h^{-1} (page 39, line 18 to page 40, line 6 of the '847 application as filed). To my knowledge, this growth rate is faster than that documented for any other *Megasphaera elsdenii* strain. The measured growth rate of CH4 directly addresses a major shortcoming within the known *Megasphaera elsdenii* strains – their relatively slow growth rate. The high growth rate of CH4 obviates the need to transfer ruminal content from healthy to ailing cows. Using CH4, a fast growing bacterial population can be introduced into the animal using a feed additive “right off the shelf” either at the time of need or as a preventive measure. Upon introduction, the lactic acid consuming biomass is immediately available to address lactic acidosis.

12. As corollary, its rapid growth rate provides CH4 with another attribute missing from previously characterized *Megasphaera elsdenii* strains. It accumulates biomass rapidly. Because of its cell density, the resultant CH4 population is much more tolerant to environmental stresses such as oxygen exposure. In order to survive, less dense populations of anaerobic bacteria must divert cellular energy to synthesize enzymes and components used to combat oxygenation effects. The consequence is reduced bacterial viability and activity. The ability of strain CH4 to grow to such high population densities that oxygen intrusion is minimized makes CH4 significantly more robust, especially for commercial production and use.

13. The second unique set of attributes are the ability of strain CH4 to metabolize lactic acid at pH values well below pH 5.5 and the ability to replicate at a measurable μ_{max} at pH 4.5 (Fig. 1 and page 39, line 8 to page 40, line 6 of the '847 application as filed). Indigenous *Megasphaera elsdenii* strains (e.g., ATCC 25940) have minimal activity level, let alone replication capability in this low pH range. The invention of the present '847 application as claimed in the presently amended claims requires a growth rate on lactate medium of at least about 0.19h^{-1} at pH 4.5 and that is at least 20% of the growth rate on lactate medium at pH 5.5, and the peak growth rate is at least 0.93h^{-1} . To my knowledge, these attributes are missing from the previously characterized strains that were available in 2002. As a result, strain CH4 continues to consume lactic acid and produce fermentation end products at pH values far below those at which other *Megasphaera elsdenii* strains cease metabolic activity.

14. Not only does strain CH4 remain active at biologically low pH values, a third unique

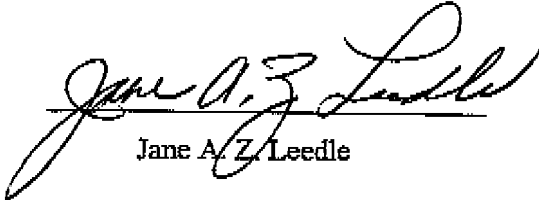
aspect (in comparison to previously characterized strains) is its ability to convert lactic acid into (primarily) acetate. A balance of acetate and propionate (fermentation end products) must be maintained for maximum productivity in ruminant animals. However, an imbalance can occur in intensively managed ruminants that are fed high grain diets. Propionate is produced in abundance from the high proportion of dietary concentrates resulting in low ruminal pH values. The lower proportion of dietary forages is fermented to acetate by fiber-degrading bacteria. But fiber-degrading bacteria are sensitive to low environmental pH values and their activity decreases. This creates a shortfall in acetate availability to the animal, which, in turn, limits production, a condition known as milk fat depression. *Megasphaera elsdenii* strain CH4 thrives at low pH values and converts lactic acid into much needed acetate.

15. In my professional opinion, after more than 50 years of searching, the ruminant agriculture industry now has a management tool for lactic acidosis that remedies the shortfalls of all *Megasphaera elsdenii* strains heretofore characterized and used as feed additives. Using an innovative screening and selection approach, the *Megasphaera elsdenii* strain CH4 was discovered and studied. For the reasons described above, strain CH4 is significantly more robust than other previously characterized strains. Strain CH4 not only utilizes lactic acid when present, but also grows fast enough to respond appropriately to changing ruminal lactic acid concentrations when livestock diets are switched from forage to grain. Strain CH4 has a high growth rate and accumulates biomass quickly thereby becoming tolerant to oxygen exposure. Strain CH4 tolerates low pH values and continues metabolic activity producing the highly desirable end product, acetate. These unique attributes were not provided by previously characterized *Megasphaera elsdenii* strains. In my professional opinion, the presently claimed invention, including, *Megasphaera elsdenii* strain CH4, and the acidosis management tool it provides, are significant advances over the state of the art at the time the invention was made.

16. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 101 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

Respectfully Submitted,

Date: February 12, 2008


Jane A. Z. Leedle

JANE A. Z. LEEDLE, Ph.D.

BACKGROUND SUMMARY

Ph.D. Microbiologist with extensive experience in anaerobic, gastrointestinal microbiology, animal agriculture, ruminant nutrition and fermentation systems; Developed new microbial products with proven influence on livestock performance; Key expertise in bacterial metabolism, microbial physiology, and product development; Experience in commercial scale up, full technical scale fermentation, downstream processing, and stability of probiotic and indigenous bacteria; R&D liaison with manufacturing personnel to solve commercial production problems. Professional experience in:

- Gastrointestinal microbiology
- Fermentation Systems
- Anaerobic waste management
- Microbial food safety
- Competitive exclusion
- Ruminant nutrition
- Beef cattle management
- Dairy cattle management
- Silage fermentation

PROFESSIONAL EXPERIENCE

JL MICROBIOLOGY, INC. Hartland, Wisconsin
President

2005 to Present

A consulting company whose mission is to help clients (1) increase their understanding of microbes and microbiological processes, (2) solve commercial microbial production problems, (3) build research and development strategies for products desired to increase fermentation and/or livestock production efficiencies, and (4) educate client personnel in the uses of microorganisms and microbial products for nutrition and health.

- New Feed Additive Applications (Direct Fed Microbial products)
- Direct Fed Microbial strain selection, product testing programs and QC
- Orphan drug research and development, fermentation and cGMP manufacturing
- Client education sessions on various microorganisms and their applications in the marketplace

CHR. HANSEN, INC. Animal Health & Nutrition, Milwaukee, Wisconsin
Director of Research, Development and Applications

1994 to 2005

- Developed three new 'Biomax' brand silage inoculant products, two new 'Probios' brand Direct Fed Microbial (DFM) products for marketplace launch, and three new DFMs as private label products for select customers
- Developed commercial process for a mixture of intestinal anaerobes and wrote majority of Chemistry of Manufacturing and Controls section for an FDA food safety drug application
- Solved fermentation issues with Human Health and Meat cultures
- Developed layman's language sales and laboratory personnel training sessions in general and rumen microbiology, silage fermentation, modes of action of lactic acid bacteria (probiotics), actions of the normal flora of the gastrointestinal tract, and developments in molecular microbiology
- Provided technical support to help "sell" high tech products to skeptical nutritionists & challenging customers

KANSAS STATE UNIVERSITY, Manhattan, Kansas
Assistant Professor, Department of Clinical Sciences; College of Veterinary Medicine

1989 to 1994

- Conducted independent research with Animal Health and Pet Food companies (ionophores, fiber)
- Taught "Rumen Physiology" to freshmen and "Comparative Animal Nutrition" to juniors

- Developed 6-week short courses for senior rotations in rumen microbiology and diagnosing and treating digestive problems in high producing dairy cows or beef cattle
- Served as consultant to local dairy farmers and beef cattle industry of Kansas, Texas and Colorado
- Developed and taught a two-day short course in Rumen Microbiology at the USDA-Meat Animal Research Center

THE UPJOHN COMPANY, (now Pfizer, Inc.) Kalamazoo, Michigan
Research Scientist II and Project Leader

1982 to 1989

- Project Leader for discovery team of 14 scientists in “Performance Enhancement: Microbiology and Physiology of Food Producing Animals”
- Discovered a strain of *Megasphaera elsdenii* to help control lactic acidosis in beef and dairy cattle
- Discovered a new rumen bacterium, *Acetitomaculum ruminis*, to reduce loss of energy to methane
- Discovered how to redirect electron flow in the pig colon to enrich for propionate production

PATENTS

Leedle, J. A. Z., S. C. Johnson, W. P. Kautz, and K. F. Lechtenberg “A farm animal product with probiotic Enterococcus bacteria.” Patent Publication No. 2005009139/WO-A1, July 30, 2004

Leedle, J. A. Z., R. C. Greening and W. J. Smolenski “Ruminal bacterium for preventing acute lactic acidosis” U.S. Patent No. 5,380,525. January 10, 1995

EDUCATION

Sabbatical	Molecular Biology & Genetics, University of Illinois at Urbana-Champaign, Illinois
Post-doc	Microbiology, University of Illinois at Urbana-Champaign, Illinois
Ph.D.	Microbiology and Dairy Science, University of Illinois at Urbana-Champaign, Illinois
MPH	Environmental and Industrial Health, University of Michigan, Ann Arbor, Michigan
BS	Fisheries and Wildlife, Michigan State University, E. Lansing, Michigan

PROFESSIONAL MEMBERSHIPS

American Society for Microbiology (ASM)
American Society of Animal Science (ASAS)
American Dairy Science Association (ADSA)
Conference on Gastrointestinal Function (CGIF)
Society of Microbial Ecology and Disease (SOMED)
American Association for the Advancement of Science (AAAS)

PROFESSIONAL ACTIVITIES

Invited Participant in The American Academy of Microbiology colloquium on Probiotic Microbes, 2005; Workshop proceedings resulted in the publication, Probiotic Microbes: The Scientific Basis, 2006.

Organizing Committee member and Secretary for the Conference on Gastrointestinal Tract Function, Chicago, IL: 2000, 2002, 2005, 2007, 2009

Ad hoc grant reviewer, USDA CSRS National Research Initiative Competitive Grants Program, Improving Animal Growth and Development Program, 1999

Ad hoc grant reviewer, USDA CSRS National Research Initiative Competitive Grants Program, Value Added Products, Non-Food Characterization, Process/Product Program, 1997

Review Panel Member, USDA CSRS National Research Initiative Competitive Grants Program, Animal Growth and Development Program, 1996

Ad hoc grant reviewer, USDA CSRS National Research Initiative Competitive Grants Program, Water Quality Program, 1993 and 1994

Review Panel Member, USDA CSRS National Research Initiative Competitive Grants Program, Alcohol Fuels Program, 1992

Ad hoc grant reviewer, National Science Foundation, Metabolic Biology Program 1986 and 1989

Editorial Board Member, Journal of Applied and Environmental Microbiology, 1989 to 1997

Program organizer for industrial sponsorship of Conference on Rumen Function, Chicago, IL 1985, 1987, 1995 and 1997

AWARDS and HONORS

Distinguished Volunteer of the Year, 1998 Outstanding community service through the Young Christian Men's Association (YMCA) of Metropolitan Milwaukee, Southwest branch; Awarded May, 1999

Distinguished Service Award – American Society for Microbiology, 1997 For serving three consecutive terms as Editorial Board Member for the *Journal of Applied and Environmental Microbiology*. 1989-1997

PUBLICATIONS

Emmanuel, D.G.V., A. Jafari, K. A. Beauchemin; **J.A.Z. Leedle**, and B.N. Ametaj. 2007. Feeding a combination of lactate-utilizing and lactate-producing bacteria modulates acute phase response in feedlot steers. *Canadian J. Anim. Sci.* 87: 251-257

Emmanuel, D.G.V., A. Jafari, K. A. Beauchemin; **J.A.Z. Leedle**, and B.N. Ametaj. 2007. Feeding live cultures of *Enterococcus faecium* and *Saccharomyces cerevisiae* induces an inflammatory response in feedlot steers. *J. Anim. Sci.* 85:233-239.

Nocek, J.E, W.P. Kautz, **J.A.Z. Leedle**, E. Block. 2003. Direct-fed microbial supplementation on the performance of dairy cattle during the transition period. *J. Dairy Sci.* 86: 331-335.

Beauchemin, K. A.; Yang, W. Z.; Morgavi, D. P.; Ghorbani, G. R.; Kautz, W.; **J.A.Z. Leedle**. 2003. Effects of bacterial Direct-Fed Microbials and yeast on site and extent of digestion, blood chemistry, and subclinical ruminal acidosis in feedlot cattle. *J. Animal Sci.* 81: 1628-1640.

Ghorbani, G.R., D.P. Morgavi, K.A. Beauchemin and **J.A.Z. Leedle**. 2002. Effects of Direct-Fed Microbials on ruminal fermentation, blood variables and microbial population of feedlot cattle. *J. Animal Sci.* 80:1977-1986.

Nocek, J.E, W.P. Kautz, **J.A.Z. Leedle**, J.G. Allman. 2002. Ruminal supplementation of Direct-Fed Microbials on diurnal pH variation and in situ digestion in dairy cattle. *J. Dairy Sci.* 85:429-433.

Leedle, J.A.Z. 2000. Intestinal Microbiology – Action Mechanisms. Alternative Feed Additives Conference Proceedings. Sao Paulo, Brazil

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LeVan, T. D., J. A. Robinson, J. Ralph, R. C. Greening, W. J. Smolenski, **J. A. Z. Leedle** and D. M. Schaefer. 1998. Assessment of reductive acetogenesis with indigenous rumen bacterial populations and *Acetitomaculum ruminis*. *Appl. Environ. Microbiol.* 64: 3429-3436.

Kung, L. Jr., E. M. Kreck, R. S. Tung, A. O. Hession, A. C. Sheppard, M. A. Cohen, H. E. Swain, and **J. A. Z. Leedle**. 1997. Effects of a live yeast culture and enzymes on *in vitro* ruminal fermentation and production of dairy cows. *J. Dairy Sci.* 80:2045-2051.

Craig, A. M., L. L. Blythe, D. E. Wachenheim, and **J. A. Z. Leedle**. 1995. Pyrrolizidine alkaloid (Tansy ragwort) degradation by ruminal microbes of sheep and longhorn cattle. *Human & Vet. Toxicol.* 62:551-555.

Leedle, J. A. Z., M. L. Coe, and R. A. Frey. 1995. Evaluation of health and ruminal variables during adaptation to grain-based diets in beef cattle. *Am. J. Vet. Res.* 56:885-892.

Leedle, J. A. Z. 1993. Modulating the Ruminal Fermentation in High-Grain Fed Cattle: The Role of Rumensin. "Scientific Update on Rumensin/Tylan for the Professional Feedlot Consultant." Chapter 2. Pages 1-24. IN: Proceedings from Symposium sponsored by Elanco Animal Health and Lilly Research Laboratories, Indianapolis, IN.

Gaines, J. D., J. Galland, **J. A. Z. Leedle**, and R.A. Basaraba. 1993. An outbreak of digital papillomatosis ("Hairy warts") in dairy cattle: Effect of the disease on production and response to surgical removal and autogenous vaccination. *Dairy Day 1993*. Kansas State University Cooperative Extension Service. pp. 46-50.

Leedle, R. A., **J. A. Z. Leedle** and M. D. Butine. 1993. Evidence that vitamin E is not ruminally degraded. *J. Anim. Sci.* 71:3442-3450.

Leedle, J. A. Z. 1991. Ration ingredients influence microflora and end products of digestion. *Animal Nutrition Research Highlights. American Soybean Association.* 91(3):1.

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Peters, J. P., **J. A. Z. Leedle**, and J. B. Paulissen. 1989. Factors affecting the *in vitro* production of volatile fatty acids by mixed bacterial populations from the bovine rumen. *J. Animal Sci.* 67:1593-1602.

Butine, T. J., and **J. A. Z. Leedle**. 1989. Enumeration of selected anaerobic bacterial groups in cecal and colonic contents of growing-finishing pigs. *Appl. Environ. Microbiol.* 55:1112-1116.

Greening, R. C., and **J. A. Z. Leedle**. 1989. Enrichment and isolation of *Acetitomaculum ruminis*, gen. nov., sp. nov.: acetogenic bacteria from the bovine rumen. *Arch. Microbiol.* 151:399-406.

Barsuhn, K., S. T. Chester, and **J. A. Z. Leedle**. 1988. *in vitro* detachment of bacteria from ruminal digesta by buffered sodium oleate solutions. *Curr. Microbiol.* 16:337-341.

Leedle, J. A. Z., and R. C. Greening. 1988. Postprandial changes in methanogenic and acidogenic bacteria in the rumens of steers fed high- or low-forage diets once daily. *Appl. Environ. Microbiol.* 54:502-506.

Leedle, J. A. Z., K. Barsuhn, and T. J. Butine. 1987. Comparison of treatments for the *in vitro* detachment of cellulolytic bacteria from ruminal digesta samples. *Curr. Microbiol.* 15:129-131.

Leedle, J. A. Z., and T. J. Butine. 1987. Enumeration of cellulolytic anaerobic bacteria from the bovine rumen: Comparison of three methods. *Curr. Microbiol.* 15:77-79.

Leedle, J. A. Z., K. Barsuhn, and R. B. Hespell. 1986. Postprandial trends in estimated rumen digesta polysaccharides and their relation to changes in bacterial groups and rumen fluid characteristics. *J. Animal Sci.* 62:789-803.

Nicoletti, J. M., C. L. Davis, R. B. Hespell, and **J. A. Z. Leedle**. 1984. Enumeration and presumptive identification of bacteria from the small intestine of sheep. *J. Dairy Sci.* 67:1227-1235.

Leedle, J. A. Z., and R. B. Hespell. 1984. Changes of bacterial numbers and carbohydrate fermenting groups during *in vitro* rumen incubations with feedstuff materials. *J. Dairy Sci.* 67:808-816.

Salyers, A. A., and **J. A. Z. Leedle**. 1983. Carbohydrate metabolism in the human colon. *Human Intestinal Microflora in Health and Disease.* D. J. Hentges, Ed. Chapter 6, pp. 129-146. Academic Press, Inc. New York.

Leedle, J. A. Z., and R. B. Hespell. 1983. Brief incubations of mixed ruminal bacteria: Effects of anaerobiosis and sources of nitrogen and carbon. *J. Dairy Sci.* 66:1003-1014.

Gomez-Alarcon, R. A., C. O'Dowd, **J. A. Z. Leedle**, and M. P. Bryant. 1982. 1,4-Napthoquinone and other nutrient requirements of *Succinivibrio dextrinosolvens*. *Appl. Environ. Microbiol.* 44:346-350.

Leedle, J. A. Z., M. P. Bryant, and R. B. Hespell. 1982. Changes in carbohydrate fermenting groups in the rumen. *Illinois Dairy Report*, Illinois Cooperative Extension Service and Agricultural Experiment Stations. pp. 28-29.

Leedle, J. A. Z., and R. B. Hespell. 1980. Differential carbohydrate media and anaerobic replica plating techniques in delineating carbohydrate utilizing subgroups in rumen bacterial populations. *Appl. Environ. Microbiol.* 39:709-719.

Leedle, J. Z. and R. B. Hespell. 1979. Responses of rumen bacteria to starvation. *Feedstuffs* 51(31):28.

Leedle, J. Z. and R. B. Hespell. 1979. Some responses of rumen bacteria to total nutrient starvation. *Illinois Research* 21:14-15.

INVITED PRESENTATIONS

"Microbial ecology-based biotechnology for the swine industry." Sponsored by the National Pork Board. St. Louis, MO, 2001

"Ensuring the Viability of Probiotic Cultures." Discover Conference on Probiotics. Sponsored by the American Dairy Science Association, Nashville, IN, 2000

"Evidence for a Microbiological Solution to Subacute Acidosis in Dairy Cows." Department of Animal Sciences, University of Wisconsin – Madison, Madison, WI, 2000

Food Safety Products - the development of MSC™ for the poultry industry". Milwaukee Rotary Club, 1999

"*Bacillus coagulans* CH-371: A New Direct Fed Microbial Product Candidate". Discovery of Novel Bacterial Strains for Aerobic Stability in Corn Silage" Second Chr. Hansen, Inc. R&D Seminar, Hørsholm, DK, 1998

"BioPlus 2B: Behind the Scenes Research and Development". First annual Chr. Hansen, Inc. R&D Seminar, Gilleleje, DK, 1996

"Discovery of Novel Bacterial Strains for Aerobic Stability in Corn Silage". First annual Chr. Hansen, Inc. R&D Seminar, Gilleleje, DK, 1996

"Ecology of acetogens in intestinal systems". Acetogenesis Symposium. American Society for Microbiology annual meeting. Washington, D.C., 1995

"Uses of ribosomal RNA-targeted hybridization probes in normal flora microbiology". Pioneer Hi-Bred International, Inc. Johnston, IA, 1993

"The role of Rumensin in modulating ruminal fermentation in high-grain fed cattle". Sponsored by Eli Lilly and Company, Lilly Research Laboratories. Amarillo, TX and Denver, CO, 1993

"Biotransformation of selected toxic compounds by the forestomach flora in the Bowhead whale". Barrow, AK. Sponsored by the Office of Naval Research in conjunction with Oregon State University, College of Veterinary Medicine, Corvallis, OR, 1993

"Characterization of the predominant microorganisms in the forestomach of the Bowhead Whale". Barrow, AK Sponsored by the Office of Naval Research. 1993

"Assessment of postprandial changes in rumen bacterial subgroups in forage- and grain-fed cattle". Oregon State University, College of Veterinary Medicine, Corvallis, OR 1993

"Rumensin and the ruminal fermentation in high-grain fed cattle". University of Nebraska, Lincoln, NE, 1993

"Acetogenesis in the gastrointestinal tract". Analytical Chemistry Laboratory group, Department of Clinical Sciences, Kansas State University, Manhattan, KS, 1993

"Genesis of Acetate: Opportunities to Reduce Methane Emissions from Livestock". Eli Lilly Company, Greenfield, IN, 1992

"Acetogenesis from Animal Guts: Problems and Challenges". Elanco Animal Health Division, Eli Lilly Company, Greenfield, IN, 1992

Utility of Ribosomal RNA Probes for Quantifying Specific Bacteria within Consortia". Department of Pathology and Microbiology, Kansas State University, Manhattan, KS, 1992

"Exploration without Cultivation: Development and Use of rRNA Probes". Department of Clinical Sciences, Kansas State University, Manhattan, KS, 1992

"Rumen metabolism and rumen microbiology". Two day short course. Great Plains Veterinary Continuing Education Center. Clay Center, NE,. 1991

"Microbiology and physiology of the rumen and its response to different feeding strategies". American Assoc. Bovine Practitioners. Indianapolis, IN, 1990

"Rumen microbiology of feedlot cattle". Central States Veterinary Conference. Kansas City, MO, 1990

"The basics of rumen microbiology in the grain fed beef steer". Academy of Veterinary Consultants. Golden, CO, 1989

"Ruminal acetogenic bacteria: their discovery and role in the hydrogen economy of the rumen microbial ecosystem". Department of Animal Science and Industry. Kansas State University, Manhattan, KS, 1989

"The postprandial ruminal ecosystem: Feed, flora, fauna and fermentation products." Mini-symposium, Guts and microbes: Gastrointestinal micro-ecology. Kellogg Biological Station. Michigan State University, E. Lansing, MI, 1986

"Diurnal changes in bacterial groups, rumen fluid characteristics and digesta carbohydrates in steers fed high or low forage diets." Department of Animal Science, Michigan State University, E. Lansing, MI, 1983